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~~NOVEL~~ COMPOSITIONS AND METHODS FOR REMOVAL OF MYCOTOXINS FROM ANIMAL FEED

This application claims the benefit of priority in provisional application Serial Number: 60/082,134, filed on April 17, 1998.

FIELD OF THE INVENTION

The present invention is directed to compositions and methods for reducing or ameliorating the absorption of a variety of mycotoxins in animal feeds, thus improving nutritional quality of the feeds and subsequent health and performance of animals consuming them. In particular, the compositions of the invention are comprised of a combination of a modified yeast cell wall extract and a clay, *e.g.*, a zeolite, bentonite, or other aluminosilicate clay. This combination has a surprising and unexpected additive binding effect for reducing mycotoxin contamination in animal feedstuffs.

BACKGROUND OF THE INVENTION

Every year a substantial percentage of the world's grain and hay supply for animal feeds is contaminated by toxins produced by invading molds. Decreased feed nutritive value and instances of animal poisoning are most often traced to growth of various species of Aspergillus, Fuserium, and Penicillium in stored grain or other feeds. Mycotoxins affect feed nutritive value, livestock performance, and animal health. Mycotoxin contaminated feeds are considerably less palatable to the animal, and the resulting decreased intake levels may

exacerbate poor performance and/or toxicity problems.

Mycotoxin formation may occur when the causative fungi grow on crops in the field, at harvest, in storage, or during feed processing; essentially whenever favorable conditions for their formation prevail. Generalizations about geographical distribution of particular types of mycotoxins are difficult due to widespread distribution of the causative fungi. However, aflatoxins and fumonisin tend to prevail in warmer climates, while cooler regions with higher moisture are subject to ochratoxin, zearalenone, vomitoxin (deoxynivalenol, DON), T2 toxin, and others. Each mycotoxin has its own particular effect, and all can be devastating. Co-contamination by one or more types of mycotoxin occurs naturally, and exerts a greater negative impact on health and productivity of livestock than contamination by individual mycotoxins.

The physical effects of mycotoxins range from reduced feed intake and poor feed conversion to a general inability of an animal to thrive. Symptoms vary according to toxin. Vomitoxin, called the feed refusal factor, affects mainly pigs. Zearalenone affects the reproductive organs of pigs and dairy cattle. Fumonisin causes a nervous disorder in horses due to its impact in the brain. Ochratoxin causes kidney damage. Poultry and pigs are sensitive to ochratoxin, whereas dairy cattle can tolerate higher levels of ochratoxin because of its biotransformation into a nontoxic form by ruminal bacteria. Aflatoxins, the most common mycotoxin, cause increased susceptibility to disease. At the organ or cellular level mycotoxins differ in their effects with severe damage done to the liver and kidney by aflatoxins and on reproductive organs by zearalenone. Other indices of mycotoxicosis include mammary gland swelling and ovarian atrophy

3

(zearalenone), oral lesions in chicks (T2 toxins), nervous system disorders and necrosis of the extremities (ergot alkaloids). Mycotoxins may also impact human health, as many are transferred into milk or meat following ingestion by the animal. For example, aflatoxins appear in milk as aflatoxin M1, a metabolite.

Acute symptoms of mycotoxicosis are often relatively easy to identify. However, chronic symptoms including slightly diminished performance and/or immunosuppression may result in greater economic losses. Traditional methods of dealing with mycotoxins include use of mold inhibitors to prevent mold growth in stored feeds. However, particularly in the livestock industries, economic circumstances force producers to find ways to use mycotoxin-contaminated feeds. Common methods have included dilution of contaminated feeds with feedstuffs known to be free of mycotoxins, physical separation to remove highly contaminated feeds, and ammoniation or heating to detoxify the feeds. These methods are labor-intensive and uneconomical, and may be ineffective against certain mycotoxins.

A more viable method of dealing with mycotoxin-contaminated feeds is to blend in substance capable of binding out the toxins, thus preventing absorption of the toxins into the animal's bloodstream. Few chemicals have proven successful enough to use commercially. Among these, use of mineral clays as binders has proven common. For example, U.S. Patent no. 5,149,549 teaches the use of a montmorillonite clay, particularly a bentonite clay, admixed with animal feeds as a mycotoxin binder. U.S. Patent No. 5,165,946 teaches the use of a montmorillonite clay in combination with a suitable sequestrant, particularly phosphate and polyphosphate salts, as a mycotoxin binder. U.S.

4

Patent no. 5,639,492 further refines the art, teaching the use of an acid-activated calcium bentonite clay admixed with animal feeds to reduce effects of mycotoxin contamination. However, clays as mycotoxin binders have significant limitations. Clays must be included in animal feeds at high levels to effect significant mycotoxin binding. Additionally, most clays have a limited binding efficacy range, binding only aflatoxins to any significant extent. Further, in domestic livestock production situations, excreted clays may cause problems with clogging of manure handling equipment. Thus, a need exists for a mycotoxin-binding agent, effective against a wide range of mycotoxins, which can be admixed with animal feeds at lower inclusion rates than is currently possible with substances commonly used to bind mycotoxins in feeds.

SUMMARY OF THE INVENTION

Accordingly, it is a primary object of the present invention to provide a method for binding and consequent inactivation of mycotoxins present in common animal feedstuffs.

It is a further object of the present invention to provide a method for binding and inactivation of mycotoxins present in animal feeds comprising a combination of a modified yeast cell wall extract and a mineral clay such as a zeolite or bentonite clay, or aluminum silicate.

Yet another object of the present invention is to provide a composition comprising a combination of a modified yeast cell wall extract and a mineral clay as described above which provides a surprising and unexpected additive binding

effect for reducing mycotoxin contamination in animal feedstuffs.

Still another object of the present invention is to provide a composition comprising a combination of modified yeast cell wall extract and a mineral clay as described above which may be admixed with animal feeds at lower inclusion rates than are required for other commonly available mycotoxin-binders suitable for inclusion in animal feeds.

Additional objects, advantages and other novel features of the invention will be set forth in part in the description that follows and in part will become apparent to those skilled in the art upon examination of the following or may be learned with the practice of the invention. The objects and advantages of the invention may be realized and obtained by means of the instrumentalities and combinations particularly pointed out in the appended claims.

To achieve the foregoing and other objects, and in accordance with the purposes of the present invention as described herein, a novel method is described for binding mycotoxins present in animal feeds. In particular, in a preferred embodiment, the invention provides a method and a composition for binding mycotoxins present in animal feed rations encompassing a modified yeast cell wall extract and aluminosilicate. The yeast cell wall is extracted from a yeast organism which can be any of a number of yeasts. The aluminosilicate is a standard commercial grade available from a variety of sources.

The compositions provided by the invention can be fed to any animal including, but not limited to, avian, bovine, porcine, equine, ovine, caprine,

canine, and feline species. When admixed with feed or fed as a supplement, the compositions with their surprisingly increased mycotoxin-binding capacity, decrease absorption or uptake of the mycotoxins by the affected animal, thereby improving performance and health, and reducing the incidence of mycotoxin-associated diseases.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based upon the surprising discovery that a yeast cell wall-derived extract in combination with a mineral clay provides an unexpected additive binding effect on mycotoxins in animal feeds. Thus, the invention provides a method and a composition for binding mycotoxins present in animal feeds utilizing a yeast cell wall extract/clay combination.

The yeast organism used for the composition of the present invention may be any of a number of edible yeasts including, but not limited to, *Saccharomyces*, *Candida*, *Kluyveromyces*, or *Torulaspora* species. In a preferred embodiment, the yeast used is *Saccharomyces cerevisiae* strain 1026. The yeast cell wall extract is obtained by methods commonly known in the art (*See*, Peppler, H.J. 1979. Production of yeasts and yeast products. Page 157 *in*: Microbial Technology & Microbial Processes, Vol.1 (2d Ed.), Academic Press).

Briefly, the yeast organism is grown following common techniques used in food-related fermentations and the beverage industries. Any of a number of common sugar-containing media, such as diluted molasses, may be used to provide a source of sugars for growth of the yeasts. Other media which may be

employed include wood sugars, sulfite waste liquor, and whey. The yeast biomass may then be separated and washed by centrifugation to yield a yeast cream.

Following separation, the organism is lysed. Any of a number of methods common in the art may be utilized to lyse the yeast organisms, including autolysis and hydrolysis. A preferred embodiment of the current invention allows the yeast organisms to autolyse at room temperature and pressure over a 12-24 hr period. A protease such as papain or any of a number of alkaline or neutral proteases may be added during the lysis phase to accelerate solubilization of yeast proteins and prevent agglutination of intracellular components. Following autolysis, the resultant yeast cell wall extract is washed several times by centrifugation to remove intracellular components and concentrate the extract. The resulting extract concentrate may be dried by any of a number of methods common in the art, including spray-drying or drum drying to form a hygroscopic, water-soluble powder.

The present invention also provides a method of enhancing and improving the mycotoxin-binding characteristics of a yeast cell wall extract comprising modification of the mannanoligosaccaride (MOS) portion of the cell wall by an alcohol shocking of the yeast organism during growth, e.g. during fermentation, resulting in a thickening of the yeast cell wall and an increase in the surface area available for mycotoxin binding of the resultant cell wall extract. Any of a number of standard commercially available alcohols may be used, including, but not limited to methyl, ethyl, and isopropyl alcohols. In a preferred embodiment of the current invention, the alcohol-shock of the yeast organism is accomplished using ethyl alcohol. The alcohol shock of the yeast organisms can

be performed by exposing the yeast organism to an environment comprising between about 5% and about 20% alcohol during growth. In a further embodiment the yeast organism is exposed to an environment comprising between 8% to 15% alcohol during growth. In a presently preferred embodiment, the yeast organism is exposed to an environment comprising between 10% and about 12% alcohol during growth.

The mineral clays used in the composition of the present invention may be any of a number of standard commercial grade clays suitable for inclusion in animal diets, including, but not limited to, zeolite and bentonite clays, or aluminosilicate. Clays may be obtained from a variety of commercial sources. In a particularly preferred embodiment, the invention comprises inclusion of aluminosilicate, available from a variety of commercial sources.

In a preferred embodiment, the composition of the present invention comprises between about 1% and about 10% aluminum silicate, and between about 90% and about 99% modified yeast cell wall extract. A preferred composition of the invention comprises from between about 4% to about 8% aluminum silicate and between about 92% and about 96% yeast cell wall extract. An especially preferred embodiment of the invention comprises from between 5% to about 7% aluminum silicate and between about 93% and about 95% yeast cell wall extract. The preferred physical form of the invention is a dry, free-flowing powder suitable for direct inclusion into animal feeds or as a supplement to a total mixed ration.

The compositions provided by the present invention can be added to any

commercially available feedstuffs for livestock or companion animals including, but not limited to, grains or pelleted concentrates. The composition provided by the present invention may be incorporated directly into commercially available pelleted feeds or fed supplementally to commercially available feeds. When incorporated directly into animal feeds, the present invention may be added to such feeds in amounts ranging from 0.25 to about 4 kilograms per ton of feed. In a preferred composition, the invention is added to feeds in amounts ranging from 0.5 to about 3 kilograms per ton of feed. In an especially preferred composition, the invention is added to feeds in amounts ranging from 1 to 2 kilograms per ton of feed. The composition contained in the present invention may be fed to any animal, including but not limited to, avian, bovine, porcine, equine, ovine, caprine, canine, and feline species.

The methods of the invention comprise increasing binding and removal of mycotoxins from animal feedstuffs, including, but not limited to, Aflatoxin, Zearalenone, Vomitoxin, Fumonisins, T2 toxin, and Ochratoxin, thereby increasing safety and nutritional value of the feed and the overall health and performance of the animal. The compositions of the invention are especially effective in increasing binding of Aflatoxin, Zearalenone, and Fumonisin compared to binding obtained with individual components of the invention alone.

The composition contained in the present invention may be added to mycotoxin-contaminated animal feedstuffs in amounts from about 0.0125% to 0.4% by weight of feed. In a preferred embodiment, the composition is added to mycotoxin-contaminated animal feedstuffs in amounts from about 0.025% to 0.2% by weight of feed. In an especially preferred embodiment, the invention is

added to mycotoxin-contaminated animal feedstuffs in amounts from about 0.04% to 0.1% by weight of feed.

Alternatively, the composition contained in the present invention may be directly fed to animals as a supplement in amounts ranging from 2.5 to 20 grams per animal per day. An especially preferred embodiment comprises feeding the composition contained in the present invention to animals in amounts ranging from 10 to 15 grams per animal per day. One of skill in the art can appreciate that the amount of the composition fed can vary depending upon the animal species, size of the animal and the type of feedstuff to which the composition is to be added.

EXAMPLES

The following examples are intended to be illustrative of the invention, and are not to be considered restrictive of the scope of the invention as otherwise described herein.

EXAMPLE 1

The following experiments demonstrate the *in vitro* mycotoxin-binding capacity of the compositions provided by the current invention. All experiments were done in aqueous solution. The specified toxins were added at concentrations of 2 μg/ml. One mg the modified yeast cell wall extract/aluminosilicate combination in a ratio of about 94% yeast cell wall extract to about 6% aluminum silicate was added to the mixture and held for one hour

with vortexing. Adsorbents were removed by centrifugation.

TABLE 1

IN VITRO BINDING OF MYCOTOXINS[a]

Mycotoxin[b]	% Bound[c]
Aflatoxin B1	95.2
Fumonisin B1	19.9
Vomitoxin	9.6
T2 Toxin	26.6
Zearalenone	44.7
Ochratoxin	8.8

[a] Binding assay carried out in aqueous media over a 1 hour incubation period. Mycotoxin concentrations were analyzed using standard HPLC procedures. Adsorbent added at 1 mg/culture tube.

[b] Toxin concentration = 2 μg/ml.

[c] Compared to adsorbent-free control cultures.

The composition provided by the present invention was most effective in binding Aflatoxin, followed by Zearalenone, T2 toxin, and Fumonisin. Binding of Vomitoxin (DON) and Ochratoxin was roughly equivalent. In similar experiments, the binding capacity of the composition provided by the present invention was tested for a range of mycotoxins in contaminated feed (TABLE 2).

Similar results were observed, except that binding of Fumonisin was more efficient than T2 toxin.

TABLE 2

IN VITRO ADSORPTION OF MYCOTOXINS IN CONTAMINATED FEED

Mycotoxin	Strong Binding (%)[a]
Aflatoxins	85.23
Zearalenone	66.66
Vomitoxin	12.58
Ochratoxin	12.49
T2 Toxin	33.39
Fumonisin	67.00

[a] Compared to nonspecific binding in negative control cultures.

EXAMPLE 2

The following experiments compare the mycotoxin-binding capacity of the composition provided by the present invention to other adsorbents. Table 3 illustrates comparative binding of mycotoxins by the present invention compared to yeast cell debris alone. Assay procedures were similar as described for data

presented in Table 1, except that mycotoxin concentrations in solution were determined using a commercially available direct competitive enzyme-linked immunosorbent assay (Veratox® Quantitative mycotoxin test).

TABLE 3

COMPARATIVE BINDING OF MYCOTOXINS[a]

	Adsorbent[b]	
Mycotoxin	Yeast Cell Wall Extract/Aluminosilicate	Yeast Cell Debris
Aflatoxin	84	24
T2 Toxin	0	5
Vomitoxin	12	0
Ochratoxin	42	0
Zearalenone	71	79

[a] Expressed as % bound compared to adsorbent-free control. Mycotoxins were added at a concentration of 2 μg/ml. Toxin concentrations were analyzed using a commercial ELISA test kit (Veratox® Quantitative mycotoxin test).

[b] Adsorbents added at 1mg per culture.

Compared to yeast cell debris alone, the composition provided by the present invention bound significantly more of all mycotoxins tested except for Zearalenone. Table 4 demonstrates a comparison of in vitro mycotoxin-binding

capacity of the composition provided by the present invention compared to other commercial binding agents.

TABLE 4

COMPARATIVE MYCOTOXIN-BINDING CAPACITIES OF VARIOUS ADSORBENTS IN VITRO

	Mycotoxin			
Adsorbent	Aflatoxin	Zearalenone	Fumonisin	Vomitoxin
Yeast cell wall extract/ aluminosilicate	95	52	45	10
Diatomaceous Earth	47	12	17	ND[a]
Aluminosilicate	58	5	5	ND

[a] Not Done.

The composition provided by the present invention showed marked improvements in strong mycotoxin binding compared to other binders tested. Similarly, the composition provided by the present invention markedly improved in vitro binding of Aflatoxin in contaminated poultry feed compared to binding by hydrated sodium calcium aluminosilicate (HSCAS) alone (TABLE 5).

TABLE 5

COMPARATIVE BINDING OF AFLATOXIN
IN CONTAMINATED POULTRY FEED[a]

	Adsorbent					
	Yeast cell wall extract/aluminosilicate					HSCAS[c]
Aflatoxin[b]	0.0125	0.025	0.05	0.1	0.2	0.4
50	33	58	83	8	26	54
100	48	58	69	14	47	78
200	51	62	79	25	65	78

[a] Results expressed as % bound compared to control without adsorbent.

[b] Parts per billion.

[c] Hydrated sodium calcium aluminosilicate.

Improvements in mycotoxin binding by the composition provided by the present invention were observed at significantly lower concentrations than were required for HSCAS.

These results show that the composition provided by the present invention, i.e. a modified yeast cell wall extract in combination with a suitable

mineral clay, provides an effective method for removal of mycotoxins in contaminated animal feeds at lower inclusion rates than are required for other commonly used binders.

The foregoing description of a preferred embodiment of the invention has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the invention to the precise form disclosed. Obvious modifications or variations are possible in light of the above teachings. The embodiment was chosen and described to provide the best illustration of the principles of the invention and its practical application to thereby enable one of ordinary skill in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. All such modifications and variations are within the scope of the invention as determined by the appended claims when interpreted in accordance with the breadth to which they are fairly, legally, and equitable entitled.

17